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The Pathology of Japanese Encephalitis *

A Review

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In his review of pathological studies on Japanese encephalitis conducted in Japan over the past 40 years, the author first discusses the findings obtained from post-mortem examinations of persons who had died of the disease, and, secondly, summarizes experimental research carried out on monkeys, horses and mice. He stresses that, although a great deal has been learned from these studies, much research is still needed, in which modern laboratory techniques give good hopes of success.

This review suggests that before the Second World War the histopathological findings in Japanese encephalitis resembled those of eastern equine encephalitis but that since the war they have been more like those of western equine encephalitis, although no definitive explanation of this phenomenon can yet be offered.

The history of Japanese encephalitis goes back to the so-called "Yoshiwara cold" in 1904; this was followed by encephalitis epidemics in 1924, 1935 and 1948. Each epidemic gave rise to considerable efforts to clarify the pathogenesis of the disease, and pathological studies on Japanese encephalitis over the past 40 years can be divided into five main periods.

The central interest of both pathologists and neuropathologists up to and after the epidemic of 1924 was the elucidation of lesions of the central nervous system. The second period began in 1933, when the Committee for Epidemic Encephalitis was established by the Japanese Association for the Advancement of Scientific and Industrial Research. Experimental studies with animals, including the selection of suitable species for experimental purposes, together with immunological research contributed much to an understanding of the visceral lesions encountered in Japanese encephalitis. The third period was after 1938, when the main interest was focused on the possible role of virus in the causation of the various lesions in encephalitis. In the fourth period, studies in the prevention of this disease were carried out, but the Second World War prevented any conclusive results from being obtained. In 1948 and subsequent years more outbreaks of encephalitis occurred in Japan, and further studies were pursued along previous lines but little progress was made. However, recent advances in electron microscopy, fluorescent antibody techniques and other fields suggest that we are now at a turning-point, and the prospects of obtaining fruitful results and a better understanding of this disease are brighter than ever before.

AUTOPSY FINDINGS IN JAPANESE ENCEPHALITIS

Pre-war studies

Mention must first be made of the work of Dr Wake (1933), who examined autopsy material from 20 persons who died of Japanese encephalitis during the 1924 epidemic in Tokyo, Nagano and Shikoku districts. There were nine cases in the acute stage (1-2 weeks), eight cases in the subacute stage (2 or more weeks), and three cases in the chronic stage (several months to several years with some permanent sequelae). Using both a quantitative and a qualitative approach, he investigated the inflammatory changes of the central nervous system in each stage of this disease. The results of his studies lead to the following conclusions.

In the acute stage, pathological changes in the central nervous system consist of areas of degeneration, including congestion, small haemorrhages,

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thrombus formation, acute and severe damage to nerve cells, neuronophagia, and injury to the cerebral parenchyma such as minute necroses, softening, and perivascular cuffing. These changes are always observed in the grey matter of the brain. The main lesions are most frequently noticed in the diencephalon and the mesencephalon. Next in frequency come lesions in the brain stem, the cerebral cortex and the cerebellum, and slight changes are occasionally seen in the leptomeninges of the spinal cord. In the subacute stage, findings consistent with inflammation and circulatory disturbance are fewer than in the acute stage, and degeneration or loss of nerve cells and proliferation of glial cells become relatively predominant. The lesions tend to be localized. Marked inflammatory changes in this stage are often found in the diencephalon, mesencephalon and brain stem; and only rarely in the cerebral cortex, cerebellum, spinal cord and meninges. In the chronic stage, degeneration or disappearance of nervous tissue, fibrous thickening of the vascular walls and post-inflammatory organization become more marked. Changes such as localized necrosis or softening are mainly observed in the mesencephalon, diencephalon and brain stem, and are especially predominant in the substantia nigra.

The main changes in the visceral organs are in the reticulo-endothelial system, the cells of which appear to be undergoing karyolysis or autolysis in the acute stage. Some cases in the acute or subacute stage show megakaryocytic embolism in the lung, liver, kidneys and spleen. In the central nervous system, megakaryocytic embolism is very rare, but hyalin thrombus formation is sometimes found in the acute or subacute stage.

Dr Wake (1933) considered Japanese encephalitis to be a generalized toxic inflammation involving not only the central nervous system but also the visceral organs, and this acute view is worth mentioning, especially when one considers that the encephalitis virus had not been discovered at that time.

The present author and T. Ogata (Miyake, 1936, 1948; Ogata & Miyake, 1935; Ogata, 1935) carried out a study of this disease about 30 years ago. From a series of 10 autopsy cases of Japanese encephalitis in Tokyo in 1935, the following histological findings were obtained: perivascular cell infiltration or cuffing, glial proliferation, glial nodules, chromolysis of Nissl's bodies, neuronophagia, haemorrhages, necrosis, softening and calcification. A comparative assessment of the distribution and localization of the cerebral lesions was also attempted. The findings in two typical autopsies are selected and presented here.

The first case is that of a 20-year-old Japanese female who was admitted to the Medical Clinic of Tokyo University Hospital with headache and general fatigue. On admission, physical examination revealed a temperature of 102°F (38.9°C), cyanosis of the lips, apathy, drowsiness, slight neck rigidity, a positive Kernig's sign, rigidity of the right leg and left hand, and leukocytosis. On the 6th day after admission to hospital, the patient died of a sudden respiratory arrest. In the brain tissue, neuronophagia was seen. Hortega cells were found around the ganglion cells, some of them in the cytoplasm of the degenerated nerve cells. There were small glial nodules consisting of proliferating Hortega cells in the areas of degenerated nerve cells, which were usually around the small vessels (Fig. 1). There were also large cell aggregates consisting of Hortega cells, oligodendroglia, and astrocytes. A diffuse proliferation of glial cells could be seen in the loosely textured background (Fig. 2). The perivascular cell infiltration or cuffs consisted mainly of lymphocytes. A rarefied elliptical area of the necrotic focus contained scattered nerve cells. Necrotic areas showing incomplete softening, in which the special staining for myelin sheath reveals shadowing of the sheath, were also seen (Fig. 3).

The pathological changes in the central nervous system in this case can be summarized as follows. Perivascular cell infiltration was marked, mainly in the telencephalon and diencephalon; and glial nodules were found in the diencephalon and midbrain, especially in the substantia nigra and nucleus ruber. There was often neuronophagia in the hippocampus, globus pallidus, and caudate nucleus. Haemorrhages occurred in the telencephalon and globus pallidus. Occasional necrotic foci were found in the caudate nucleus, but they were few in number.

The second case is that of a 16-year-old Japanese female who was admitted to the Medical Clinic of the Tokyo University Hospital with fever and marked fatigue. On admission, she had a temperature of 101°F (38.3°C), lethargy, apathy, severe headache and neck rigidity. On the following day, she fell into a stuporous state, and marked rigidity in the neck and left extremities was noticed. She then developed tremor of the right hand. She died on the 12th day in hospital.

Post-mortem examination revealed necrotic areas in the brain with incomplete softening and infiltration of neutrophils, minute haemorrhagic areas in the brain substance, and also haemorrhage into the Virchow-Robin space. There was hyalin thrombus formation in the cerebral vessels, as Wake (1933) pointed out about 30 years ago (Fig. 4). The main pathological features of the central nervous system may be summarized as follows. Perivascular cell infiltration was fairly marked, mainly in the telencephalon and diencephalon. In the telencephalon and midbrain, there were glial nodules. Diffuse

proliferation of glial cells occurred from the mesencephalon to the olivary nucleus. Neuronophagia was found in the thalamus, pallidum, and caudate nucleus. In addition, there were haemorrhages in the pallidum.

Among the changes in the visceral organs in this disease, one in the lung was first described in 1935 by Dr Ogata and myself and we have continued our observations on it since that date. A peculiar feature of this alteration is increased cellularity of the alveolar septa, which Tanabe (1936) called " interalveolitis ". He emphasized the fact that fatty degeneration of the elastic fibres of the alveoli in the lung was a specific feature among epidemic encephalitis cases. As the term " interalveolitis " indicates, the pneumonia-like cellular exudation into the alveolar lumina is absent, and Kawamata (1940, 1941) proposed the term " pneumonia interstitialis alveoloseptica ". Haemorrhage into the alveolar lumina, if accompanied by secondary infection, tends to be followed by catarrhal pneumonia as a complication. There is a minimal inflammatory reaction in the visceral organs—namely, hyperplasia of the reticuloendothelial cells in the spleen, liver, and lymph-nodes, and diffuse or nodular hyperplasia of the mononuclear cells in the cardiac interstitial tissues. These findings suggest a viraemia or general dissemination of virus through the visceral organs before any clinical manifestation of the inflammatory process appears in the central nervous system.

The following case-history may be taken as demonstrating typical visceral changes in Japanese encephalitis. A 27-year-old Japanese female first complained of headache, fever—she had a temperature of 104.9°F (40.5°C)—and vomiting. Two days after the onset of the disease, she became lethargic and complained of abdominal pain. She was admitted on 6 August 1958 to the Paediatric Clinic of the Komagome Hospital. On admission, no Kernig's sign, no neck rigidity, and no Babinski's sign was noticed. On the sixth day after admission, a convulsive seizure occurred. This was followed by a severe stridor and she expired on the seventh day in hospital. The lung showed partial thickening of the alveolar wall, disclosing moderate cellularity. Fig. 5 is suggestive of interalveolitis. In the spleen, the Malpighian bodies showed slight hyperplasia of the reticulum cells, which occasionally were in a degenerating state. In the heart, there was a more or less increased number of perimysial mononuclear cells; and perivascular oedema was observed (Fig. 6).

Dr Kawakami (1935) examined autopsy material from 34 persons who died during the 1935 epidemic in Tokyo. From the histopathological changes in the central nervous system, he emphasized the importance of softening and discussed the relation between softening and glial aggregates.

Post-war studies

After the Second World War, when Japanese encephalitis broke out in the Tokyo and Osaka areas, Dr Shiraki (1962) examined the brains in autopsy cases; some patients had died in the fulminating stage, after 3-5 days, six in the acute stage, after 6-9 days, and seven in the protracted stage, after 11-50 days. Perivascular cell cuffs were characteristic morphological findings of the fulminating and acute stages. Cellular nodules and rarefied necrotic foci occurred within the grey matter. Perivascular cuffing and cellular nodules were found scattered widely from the cerebrum, diencephalon, cerebellum and brain stem to the spinal cord; and were especially marked in the cerebral cortex, thalamus, and substantia nigra. Rarefied focal necrotic areas were frequently visible to the naked eye on the autopsy table. They were often located in intimate relation to the arterioles, surrounded by perivascular cell cuffs. Shiraki (1962) also described several subtypes of rarefaction focal necrosis. On the other hand, haemorrhage, glial shrub formation and pseudolaminary cortical necrosis were rare findings in the fulminating and acute stages. In the protracted stage, there was abundant cellularity in the rarefied necrotic foci. The cell component consisted of active rod-shaped cells and gitter cells, laden with neutral fat granules derived from the breakdown products of the myelin sheath. Occasionally, there was deposition of fine calcareous granules in the necrotic nerve cells, parenchyma, etc. With reference to Shiraki's description (1962), it may be said that functional and focal vascular spasm of the intracerebral arterioles plays a significant role in the process of rarefaction necrosis formation.

A comparison may be made between various types of mosquito-borne encephalitis and Japanese encephalitis, the pathological picture described by Haymaker in 1961 being quoted. The lesions in eastern equine encephalitis are predominantly in the cerebral cortex, thalamus, caudate nucleus, substantia nigra, etc. Perivascular cell infiltration and glial nodule formation are common histological features. In western equine encephalitis, the characteristic lesion is rarefaction necrosis, the distribution of the lesions being similar to that in the eastern type. The lesions in St Louis encephalitis are mainly in the

thalamus and substantia nigra. Their features are perivascular cell cuffing and glial nodule formation. When Japanese encephalitis as reported by Shiraki (1962) is compared with that described by Haymaker (1961), it will be observed that the difference in their histological findings lies in the evidence of rarefaction necrosis formation adduced by Shiraki. Rarefaction necrosis as discussed here was not frequently observed before the 1948 epidemic in Japan. Whether this difference is the result of variations in viral strains or in individual responses remains to be answered.

Dr Takeya (1962) of the Neuropathological Laboratory of the Neuropsychiatric Clinic, Kyushu University, examined Japanese encephalitis autopsy material in Fukuoka district during the years 1958-59. He described the pathological findings in the central nervous system as perivascular cell infiltration, localized glial proliferation (including glial nodules), neuronophagia and glial shrub formation, and necrosis, softening, haemorrhage, pseudolaminary cortical necrosis, and calcification of the capillary walls. Two of his cases will be described.

The first case is of a 10-year-old Japanese female admitted to the Paediatric Clinic of the Kyushu University Hospital. On admission, she had a temperature of 101.8°F (38.8°C), neck rigidity, Kernig's sign, and leukocytosis. She went into coma and died on the fourth day in hospital. Histological examination revealed circular areas of necrosis scattered throughout the grey matter of the pre- and post-Rolandic convolutions. The thalamus contained round rarefied necrotic areas, which Takeya (1962) has called pale necrosis. In other areas of the brain, there were multiple cortical and subcortical round pale foci. In the cerebellum, irregular frayed pseudolaminary cortical necrosis of vascular origin was observed (Fig. 7). Typical glial shrub formation occurred in the cerebellar cortex (Fig. 8). Some areas in the brain contained nodular cell aggregates consisting of plump microglial cells. The predominant feature in the central nervous system may be summed up as rarefaction necrosis.

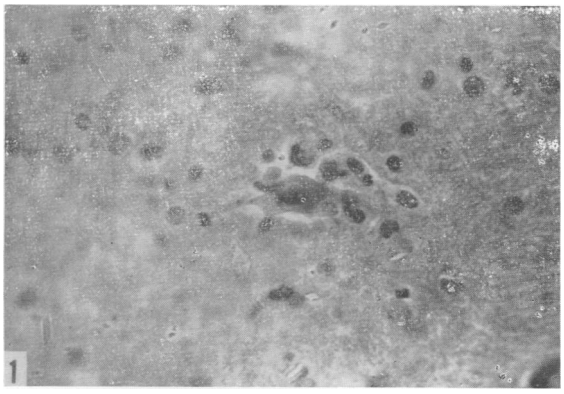
The second case is of a 7-year-old Japanese female with vomiting, headache, and lethargy beginning 8 days prior to death. A few days after onset, she was observed to have a temperature of 104°F (40°C) and mydriasis. The day after the onset of the disease, she was admitted to the Paediatric Clinic of Kyushu University Hospital with fever and pain in the neck. On admission, she had neck rigidity, Kernig's sign, tremor of the extremities, impaired light reflexes, an over-active knee jerk, and leukocytosis. She continued to run a temperature of 104.2°F (40.1°C) and then went into coma with dyspnoea, dying on the eighth day in hospital.

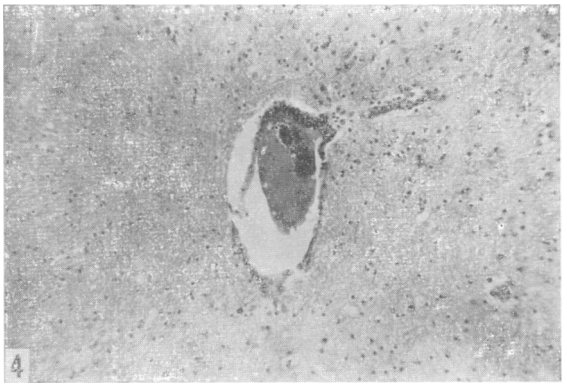
Microscopic findings in the brain were of dense perivascular infiltration throughout the cerebral tissue. There were numerous nodules in the motor cortex, and in the substantia nigra there could be seen considerable depigmentation of the ganglion cells, perivascular cuffing and glial nodules (Fig. 9). The fifth ganglionic cell layer of the pre-Rolandic convolution contained minute glial nodules. Betz's giant cells remained relatively intact (Fig. 10). In the cerebellum, glial shrub formation and diffuse microglial activity were found in the cerebellar molecular layer. The anterior horn motor cells of the cervical spinal cord revealed far advanced neuronophagia. Areas of cellular loss, suggestive of perivascular liquefactive necrosis, were found in the occipital convolutions. Microscopically the characteristics of this case were glial nodule formation and perivascular cell infiltration.

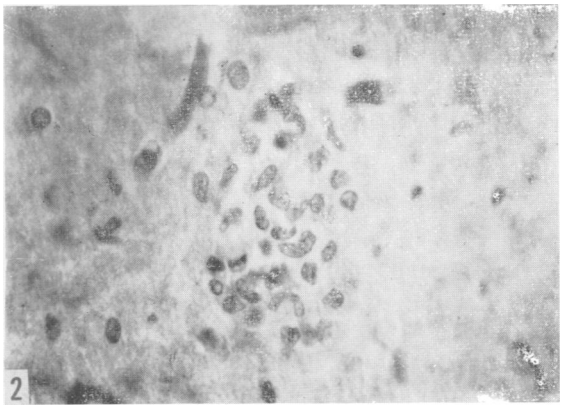
Several of the above findings require some further explanation. Takeya (1962) divides localized necrosis into dense or cellular and pale or rarefaction necrosis. There are two views on the origin of localized rarefaction necrosis in Japanese encephalitis. One, that of Matsuyama and others, is that the direct action of the virus causes cerebral necrosis developing into large confluent lesions, and the other, held by Wake (1933), Suwa and others, is that the necrosis is of vascular origin, due to a circulatory disturbance that causes localized oedema of the cerebral parenchyma around small arteries. With regard to local rarefaction necrosis and softening, Takeya (1962) has demonstrated that fat granule cells fill the softened areas, while they are absent in the rarefied areas. In some cases of Japanese encephalitis, capillary bleeding is noticed predominantly alongside perivascular cell infiltration and glial nodules. The form of the disease with severe bleeding may be called haemorrhagic encephalitis. Takeya (1962) concludes his description by saying that Japanese encephalitis might be called diencephalomesencephalitis because of the distribution of the lesions, which are found primarily in the thalamus and secondarily in the substantia nigra.

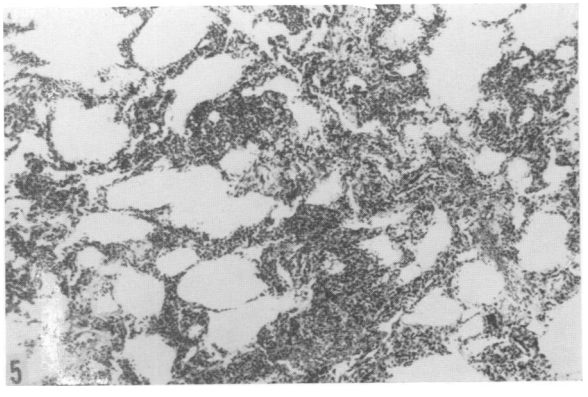
EXPERIMENTAL STUDIES ON JAPANESE ENCEPHALITIS

Before 1938, rabbits were mainly used for immunological studies, but it soon became apparent that they were not suitable because the neuropathological changes in rabbits inoculated with virus were in the ganglion or parenchymatous cells and there was minimal formation of glial nodules and perivascular infiltration. Cats were likewise found unsuitable, although they had been employed in many experi-









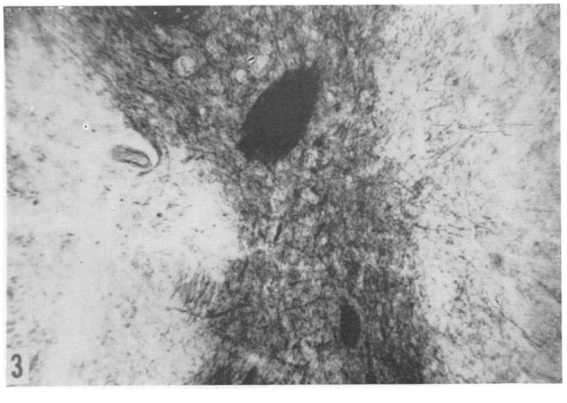


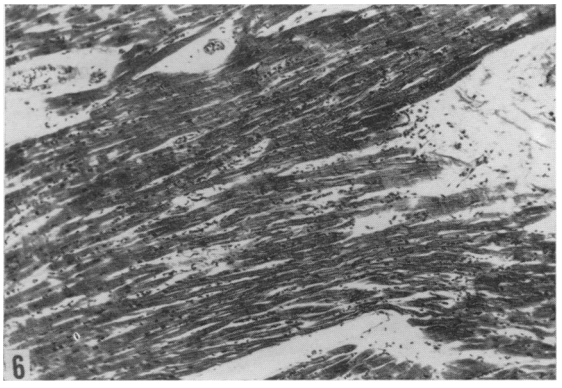
FIG. 1. 20-year-old female (Miyake's case)
Neuronophagia showing Hortega cells around ganglion cells in the brain.

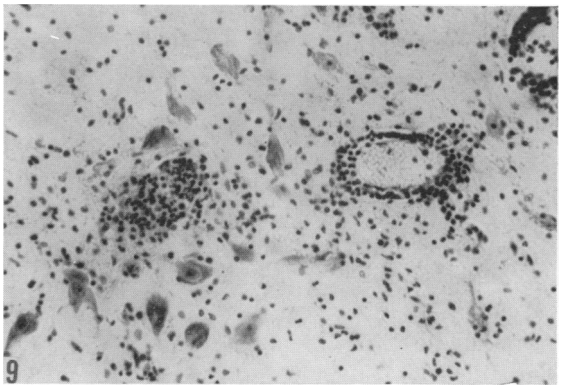
FIG. 2. 20-year-old female (Miyake's case)
Glial nodules in the brain consisting of Hortega cells.

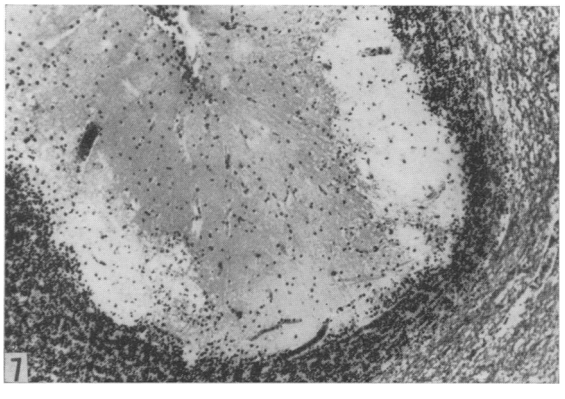
FIG. 3. 20-year-old female (Miyake's case)
Shadowing of myelin sheath in an area of softening of the brain.

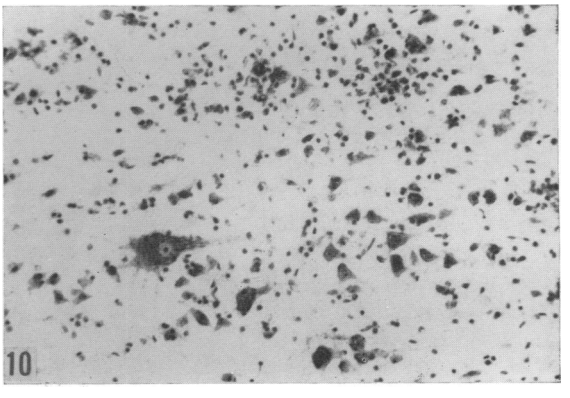
FIG. 4. 16-year-old female (Miyake's case)
Hyalin thrombus formation in a cerebral vessel.

FIG. 5. 27-year-old female (Miyake's case)
Interalveolitis of the lung, showing partial thickening of the alveolar wall and moderate cellularity.









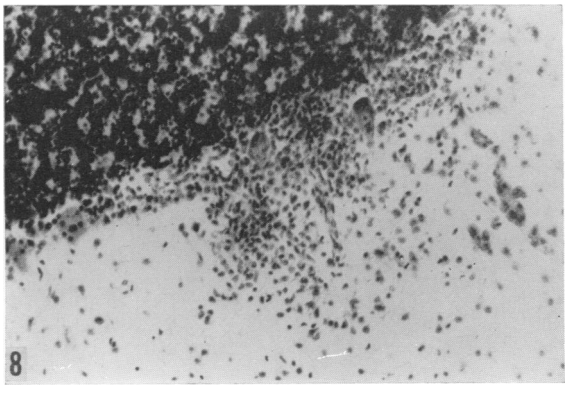


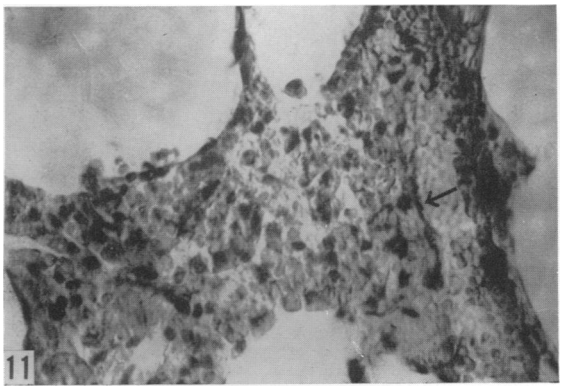
FIG. 6. 27-year-old female (Miyake's case)
Heart: perimysial mononuclear cell proliferation and perivascular oedema.

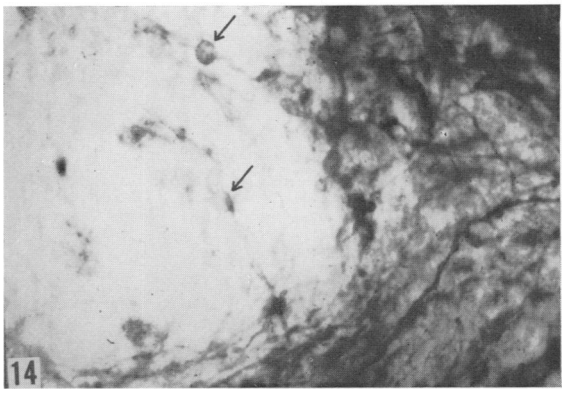
FIG. 7. 10-year-old female (Takeya's case)
Cerebellum; relatively irregular frayed necrosis chiefly confined to the molecular and Purkinje cell layers. Diffuse microglial proliferation with interfascicular swelling of the cerebellar white matter.

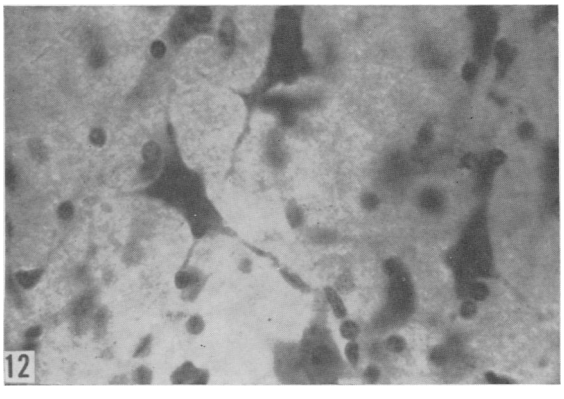
FIG. 8. 10-year-old female (Takeya's case)
Cerebellum: glial shrub formation and diffuse microglial activity in the cerebellar molecular layer.

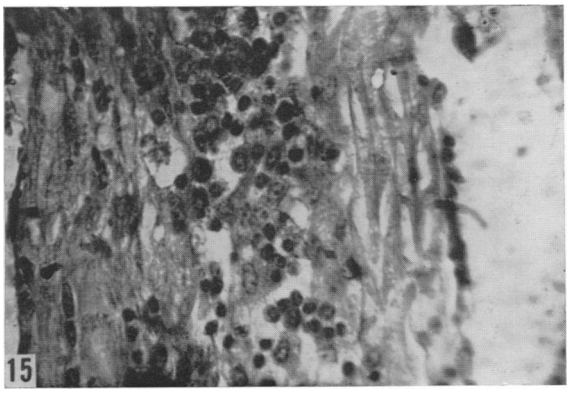
FIG. 9. 7-year-old female (Takeya's case)
Substantia nigra: diffuse microglial activity (rod cell type) and perivascular cuffing with lymphocytes and adventitial cells. Note the depigmentation in the substantia nigra.

FIG. 10. 7-year-old female (Takeya's case)
Brain: minute glial nodules in the fifth ganglionic cell layer of the pre-Rolandic convolution. Betz's giant cells remain relatively intact.









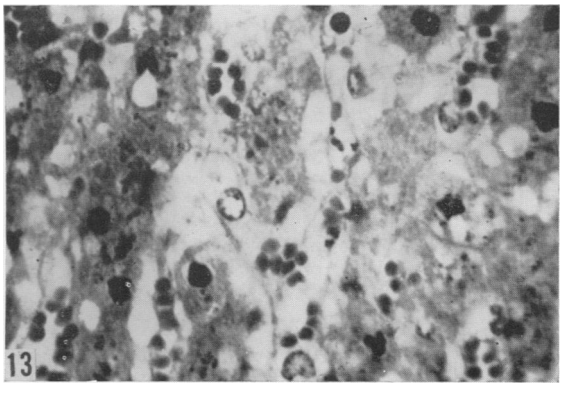


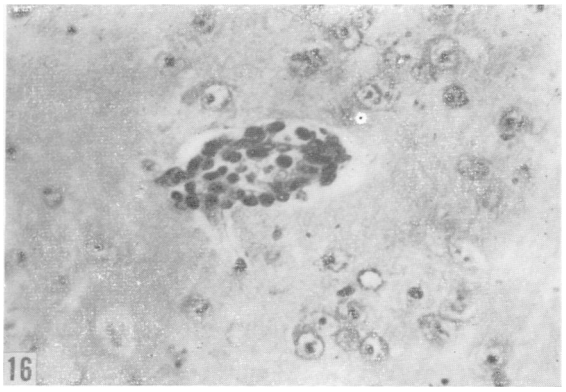
FIG. 11. Horse dying during incubation stage (Miyake's experiment)
Lung: elastic fibres destroyed in the thickened alveolar wall. The arrow indicates intact elastic fibres remaining.

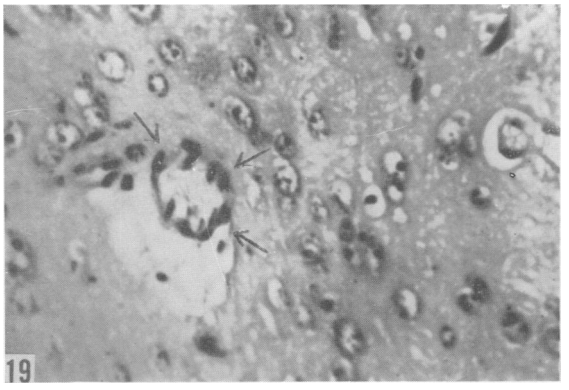
FIG. 12. Unvaccinated control horse dying during incubation stage (Miyake's experiment)
Brain: neuronophagia in the sixth cortical layer.

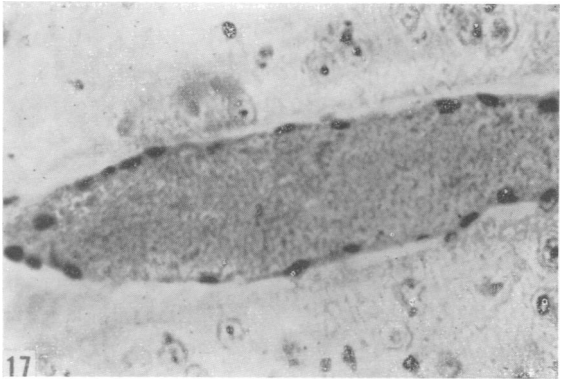
FIG. 13. Unvaccinated control horse dying during incubation stage (Miyake's experiment)
Liver: vacuolization of nuclei in swollen Kupffer cells, with some karyorrhexis.

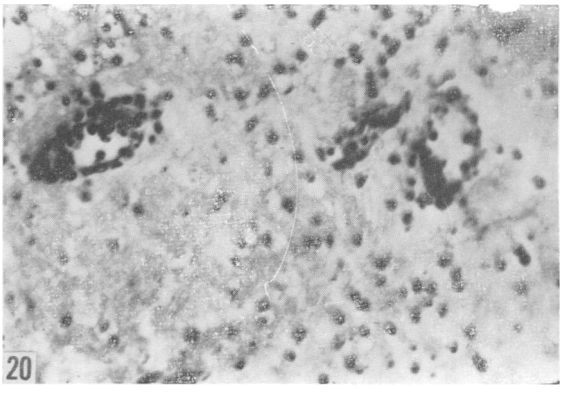
FIG. 14. Unvaccinated control horse dying on ninth day (Miyake's experiment)
Brain: globular swelling of the axis cylinder in the demyelinated area, shown by arrows.

FIG. 15. Immunized horse dying on 13th day (Miyake's experiment)
Brachial nerve: marked histiocytic infiltration into brachial nerve bundles. ("Séptinévrite" of Nicolau et al., 1929.)









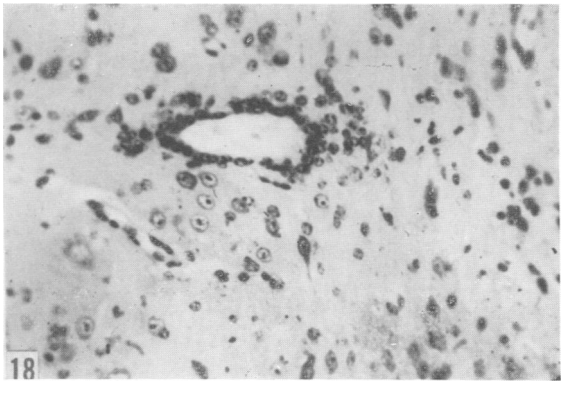


FIG. 16. Mouse group given virus; 6 days (Miyake's experiment)
Brain: perivascular cell infiltration.

FIG. 17. Mouse group given polysaccharide; 6 days (Miyake's experiment)
Brain: marked dilatation of the capillary with stasis.

FIG. 18. Mouse group given polysaccharide and virus; 7 days (Miyake's experiment)
Pons: perivascular infiltration and concomitant gliosis.

FIG. 19. Mouse group given hyaluronidase; 1 day (Miyake's experiment)
Pons: marked dilatation of both the capillary and the Virchow-Robin space. Note liquefaction of the membrana limitans gliae. The arrows indicate double-wall formation in the capillary.

FIG. 20. Mouse group given hyaluronidase and virus; 6 days (Miyake's experiment)
Brain: marked perivascular cell infiltration, gliosis, and vacuole formation.

mental studies in the past. In 1933, Webster in the USA had succeeded in inducing marked inflammation in the brain of mice, and Japanese researchers subsequently began to use mice for the inoculation of encephalitis virus. It is noteworthy that, during the same period, Hayashi (1935) in Japan succeeded in transmitting Japanese encephalitis virus to monkeys. It was later found that monkeys and horses were suitable for histopathological studies of experimental Japanese encephalitis and for investigation of virus propagation within an animal body.

Experiments on monkeys

In 1938, experimental research was conducted on monkeys by Ogata, Miyake & Takaki (1938; see also Takaki, 1943). Monkeys, inoculated paranasally, subcutaneously, intracerebrally or intravenously, showed the same histological picture as man. In the central nervous system, cell infiltration in the leptomeninges, congestion and haemorrhage in the cerebral parenchyma, perivascular cell infiltration, reactive glial proliferation, neuronophagia, acute swelling of the nerve cells, glial shrub formation, cells containing granules of fat around the perivascular spaces and pseudolaminary necrosis are found. In the visceral organs, a section of the heart shows interstitial myocarditis. The virus thus appears to have considerable affinity for the heart muscle as well as for the central nervous system. In the lung, there are oedema, haemorrhages, peribronchiolitis, interalveolitis and megakaryocyte embolism in the alveolar walls. The main histological changes in the spleen are reactive hyperplasia and degeneration of the germinal centres and an increased exudate which finally becomes hyalin material. The changes both in the visceral organs and in the central nervous system of the monkey are similar to those in man; and there is a definite chronological order in their localization and in their affinity for particular organs, the visceral phase preceding the cerebrospinal phase. These findings suggest that the virus proliferates to some extent in the visceral organs during the visceral phase and in the cerebrospinal organs during the cerebrospinal phase. This phenomenon also gives an important clue to the spread of the virus in the human body in Japanese encephalitis.

Experiments on horses

Using horses, Kii et al. (1944) and Miyake (1949) studied the clinical course of encephalitis and histopathological findings in the cerebrospinal system and the visceral organs. For these experiments, horses were imported from Hokkaido district, where equine epidemic encephalitis had seldom been observed. They were immunized by the subcutaneous injection of 10-30 ml of formol vaccine after confirmation had been obtained that their sera contained no neutralizing antibody, and were then inoculated with mouse-brain material containing equine encephalitis virus. Table 1 shows the neutralizing antibody after inoculation. The horses were divided into three groups: those dying during the incubation stage, those dying after inoculation and showing brain involvement, and those not dying from the challenge but sacrificed. The results of these experiments are summarized in Table 2. In the group dying during the incubation stage, the serological examination revealed no neutralizing antibody in the serum (horse 7). The characteristic features of this group are those of marked exudation and degeneration in the central nervous system as well as in the visceral organs. The histological examination of this group revealed marked perivascular cell infiltration in the meninges and leukocytic infiltration of the perivascular spaces in the brain. In the lung there was interalveolitis,

TABLE 1

DEGREE OF NEUTRALIZING ANTIBODY AFTER INOCULATION OF INDICATED QUANTITY OF FORMOL VACCINE

Vaccine dose / Horse No. / Date	10 ml		20 ml		30 ml	5-10 ml		Untreated control	
	1	2	3	4	5	7	8	9	10
16/12/42	—	—	—	—	—	—	—	—	—
19/12/42						Vaccinated			
26/12/42	—	—	—	—	—	—	—	—	—
	Vaccinated								
2/1/43	—	—	—	—	—	—	—	—	—
9/1/43	—	—	—	—	—	—	±	—	—
10/1/43	—	—	—	—	—	—	±	—	—
	Inoculated								
23/1/43	±	±	+	++	+		+		±
30/1/43		+	+	++	+				
6/2/43		+	+	+++	+				

TABLE 2
SUMMARY OF HORSE IMMUNIZATION EXPERIMENT WITH FORMOL VACCINE

Autopsy No.	Horse No.	Autopsy date	Day of disease [a]	Vaccine dose (ml)	Neutralizing antibody	Brain symptoms	Viraemia	Proof of virus from CNS	Stage of incubation (days)
1	7	17/1/43	2 (D)	5-10	—				
2	9	19/1/43	4 (D)	Not treated	—				
3	10	24/1/43	9 (D)	Not treated	±	+	+	+	4
4	1	28/1/43	13 (D)	10	±	+	—	+	11
5	8	28/1/43	13 (D)	5-10	+	+	+	—	11
6	5	5/2/43	21 (S)	30	+	—	—	—	11
7	3	5/2/43	21 (S)	20	+	—	—	—	11
8	2	6/2/43	22 (S)	10	+	—	—	—	11
9	4	6/2/43	22 (S)	20	+++	—	—	—	11

[a] D = Died; S = Sacrificed.

and special staining for elastic fibres revealed destruction of these fibres in the thickened alveolar wall (Fig. 11). In the lymph-nodes, moderate hyperplasia of the germinal centres was found. The spleen contained slightly enlarged Malpighian bodies, whose cells were separated by exudate. Occasional karyorrhexis was seen. In the heart, there was interstitial myocarditis, as in the experiment on monkeys by Ogata, Miyake & Takaki (1938). In an unvaccinated control animal (horse 9), meningeal changes were milder. However, cortical damage appeared to be more marked. The brain showed neuronophagia (Fig. 12). The liver contained swollen Kupffer cells, some of them showing karyorrhexis (Fig. 13). In the other control (horse 10), ischaemic and sclerotic change in the ganglion cells was observable in the central nervous system. In some areas of the brain there were shadowing of the myelin sheaths and globular swelling of the axis cylinder in the demyelinated areas (Fig. 14). A section of the lung revealed interalveolitis, impregnation with silver showing poor argentaffine fibres in the alveolar wall. This suggests histiolytic change combined with exudation and degeneration. In some areas of interalveolitis, there was megakaryocytic embolism in the alveolar wall. The spleen showed atrophic Malpighian bodies with slight fibrosis.

Turning to the immunized horses, we may take as an example horse 1, which died on the 13th day, at which time the serological examination revealed a slightly positive neutralizing antibody titre. Generally speaking, in this group there was a tendency to proliferative change in the affected organs. On the other hand, the features of degeneration and exudation were suppressed. Histological examination of the brain in this group showed typical perivascular cell infiltration, composed mainly of lymphocytes. Focal vascularized areas contained a slight proliferation of glial cells. In the hyoglossus nucleus in one horse, a glial nodule contained several leukocytes. In the liver, a miliary granuloma was observed. The lymph-node contained slightly atrophic germinal centres with amorphous material around the arterioles. In the lung, which showed interalveolitis, there was slightly increased affinity for silver stain in the fibres of the alveolar wall. In the other horse dying on the 13th day after inoculation (horse 8), the cerebral cortex contained many glial nodules and a section of a brachial nerve bundle showed marked histiocytic infiltration (Fig. 15). These findings have been called " séptinévrite " by Nicolau et al. (1929). Horse 4, autopsied on the 22nd day after inoculation, had a markedly increased neutralization antibody titre. The characteristic change in this group was proliferation. In the brain of this horse, the nerve cells presented no unusual aspect, but there was a proliferation of swollen oligodendroglia around the ganglion cells. In the thalamus, glial nodules and perivascular cell infiltration were noticeable. The spleen contained slightly hyperplastic Malpighian bodies, suggesting regenerative activity. A section of the lung revealed healed interalveolitis. As compared with the controls the changes in immu-

nized horses are more proliferative in nature and more circumscribed in extent, and the process of recovery may be observed.

Experiments on mice

Miyake et al. (1954) and Ogawa (1958) have confirmed experimentally that the capsular polysaccharide of pneumococcus 2 (*Diplococcus pneumoniae*) suppresses infection with Japanese encephalitis virus (Nakayama strain) in mice and that the spreading factor in hyaluronidase promotes virus infection. The experiments were performed on mice, some receiving a subcutaneous injection of a very small amount (0.5-1.0 μg) of pneumococcal capsular polysaccharide and some being given a subcutaneous injection of hyaluronidase—500 viscosity units dissolved in 1 ml isotonic NaCl solution (v.u.m.).

Characteristic findings in a group of mice injected subcutaneously with encephalitis virus only were slight perivascular infiltration and capillary stasis (Fig. 16). A very mild degree of degeneration in the nerve cells and of gliosis was observed. The main morphological findings in a group treated with pneumococcal capsular polysaccharide only were dilatation of the venous or capillary system and disappearance of the Virchow-Robin spaces (Fig. 17). In a group given polysaccharide followed by subcutaneous administration of virus, some of the changes found in the virus group were markedly depressed by the influence of the polysaccharide and only appeared to a slight degree in 6-8 days. Perivascular infiltration was with monocytes and degeneration of the ganglion cells was never found (Fig. 18). In a group receiving a subcutaneous injection of hyaluronidase only, marked engorgement of the Virchow-Robin space, thinning of the capillary wall, destruction of the membrana limitans gliae and vacuole formation in the parenchyma characteristically occurred. These changes were most marked on the first or second day of the experiment. Destruction of the membrana limitans gliae and double wall formation in the capillary were seen in association (Fig. 19). In a group given hyaluronidase followed by virus, the changes were much less in the initial stage, but on the fourth day severe inflammation began and the severity of the damage was about twice as much as that observed in the group given virus only. Understandably, the death-rate increased in this group (Fig. 20). These histopathological findings suggest that polysaccharide strengthens the blood-brain barrier but that hyaluronidase destroys it. The findings also suggest that the blood-brain barrier is one of the important factors in the pathogenesis of encephalitis infection, while pneumococcal capsular polysaccharide may have some influence on the metabolic conflict between host cell and virus.

CONCLUSION

From the studies reviewed above, there seems to be some relation between Japanese encephalitis as it occurred before the Second World War and eastern equine encephalitis; and also between Japanese encephalitis as it occurred after the Second World War and western equine encephalitis in so far as the main histological changes are concerned, these being characterized by rarefaction necrosis. Whether this shift in relationship is due to differences in viral strains or to variations in individual response, or to other causes, is a question that has still to be answered.

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RÉSUMÉ

L'histoire de l'encéphalite japonaise remonte à 1904 (mal de Yoshiwara); depuis lors, des épidémies sont survenues en 1924, 1935 et 1948. Les études ont progressé en cinq étapes: études post-mortem des lésions du système nerveux central; dès 1938, à la suite de la création d'un comité spécial, études sur l'animal et études immunologiques en relation avec les lésions viscérales; hypothèse d'infection virale; tentatives de prévention, interrompues par la guerre; progrès actuels dus aux techniques modernes d'exploration.

L'auteur passe en revue les découvertes histo-pathologiques des divers chercheurs, ainsi que les résultats des expériences sur les animaux (singe, cheval, souris). Dès 1933, l'encéphalite japonaise est considérée comme une inflammation toxique généralisée affectant le système nerveux et les viscères. En 1940, les caractères d'une virémie affectant les organes viscéraux avant qu'apparaissent les manifestations inflammatoires du système nerveux central sont reconnus.

La comparaison histo-pathologique entre l'encéphalite japonaise et diverses formes d'encéphalite transmises par les moustiques permettent de rapprocher de l'encéphalite équine de l'est (EEE) l'encéphalite japonaise des épidémies antérieures à 1948. La présence du caractère neuropathologique de nécrose par raréfaction, peu fréquent avant 1948, permet de rapprocher, à partir de cette date, l'encéphalite japonaise de l'encéphalite équine de l'ouest (WEE). Il n'est pas possible de dire si cette différence correspond à une variation de la souche virale ou à celle de la réponse individuelle à l'action du virus. Les études à ce sujet se poursuivent.

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